CO-AGGLUTINATION BETWEEN *B. TYPHOSUS* AND *B. PARATYPHOSUS* B

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CONTENTS

Introduction

According to Weil & Felix (1920) and to Felix (1924) group or co-agglutination in the enteric fevers is caused exclusively by the somatic ("O") agglutinins. The recorded results of recent investigators, however, show co-agglutination between the flagellar ("H") as well as the somatic ("O") agglutinins of *B. typhosus* and *B. paratyphosus* B, but its occurrence is not emphasized.

Browning (1933), who has observed co-agglutination in repeated samples of sera from carriers, refers to the scanty prominence which has been accorded its study.

Smith (1932) reported the serological and bacteriological findings in twenty-eight cases of typhoid fever and in forty-two cases of paratyphoid B fever. From a study of his Table II (p. 146) it is found that typhoid sera co-agglutinated Paratyphoid B "O" twice, Paratyphoid B "H" four times and both Paratyphoid B "H" and Paratyphoid B "O" three times. In six of these nine clinical cases of typhoid fever, *B. typhosus* was isolated from the blood or excreta. Similarly from his Table V (p. 148) paratyphoid B sera were found to co-agglutinate Ty "O" eight times, Ty "H" seven times and both Ty "H" and Ty "O" seven times. In thirteen out of these twenty-two clinical cases of paratyphoid B fever, *B. paratyphosus* B was isolated from the blood or excreta.

Smith regards these cases as examples of group agglutination, although he does not call attention to the varieties of co-agglutination as shown in his tables, nor does he state whether enquiry had been made regarding past enterica infection or previous inoculation.

Horgan (1932) examined the agglutination titres of fourteen bacteriologically proved cases of typhoid fever. Case 1 of his Table III (p. 526) shows group agglutination by typhoid sera of both Paratyphoid B "H" and Paratyphoid B "O", the various titres being Ty "H" 1 : 2500, Ty "O" 1 : 500,

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Paratyphoid B "H" 1 : 125 and Paratyphoid B "O" 1 : 125. Presumably this case was not previously inoculated, since two cases in the table are noted as having had previous T.A.B. inoculation.

TECHNIQUE

In performing agglutination tests to ascertain the end-titres of sera from the typhoid-fever cases, separate flagellated ("H") and somatic ("O") suspensions as supplied by the Standards Laboratory, Oxford, were employed. The results obtained have been recorded as the highest dilutions in which definite agglutination occurs and also as reduced titres. The latter method enables a comparison to be made when successive serum samples from the same patient are examined.

The emulsions used in the absorption of agglutinins were prepared from Felix's typhoid and paratyphoid B strains—Ty H_{901}, Ty O_{901}, HB_2 and OB, the last strain being Schütze's permanent "O" variant of a strain of *B. aertrycke*. From agar-plated cultures of these stock strains, smooth colonies were selected and the motile organisms—Ty H_{901} and HB_2, grown through semi-solid agar in motility tubes.[1] Roux bottles were inoculated with 18-hour peptone-water cultures of the four strains, the growths washed off with 3–5 c.c. of buffer saline and transferred to graduated centrifuge tubes. After centrifuging and washing twice with buffer saline, the bacteria were centrifuged until constant in volume, the supernatant saline being discarded. (The supernatant fluid was at first retained and used as antigen in the agglutination tests, but later the Oxford bacillary suspensions were used.)

To the packed bacterial mass, undiluted serum and saline were added so that the final dilution of serum and saline was 1 : 10. For optimum absorption the volume of the packed bacterial mass to the final volume of diluted serum should be 1 : 10. The formulae used by Krumwiede *et al.* (1925) for this calculation, in which a correction factor is used for the saline included in the bacterial mass are:

(1) (Mass − 10 per cent) × dose factor = total volume.

(2) $$\frac{(\text{Mass} - 10 \text{ per cent}) \times (\text{dose factor} - 1)}{\text{serum dilution factor}} = \text{amount undiluted serum required.}$$

In an actual experiment:

The packed mass of centrifuged bacteria = 0·9 c.c. (absorbing dose).

10 per cent correction for residual saline $= \frac{0{\cdot}09 \text{ c.c.}}{0{\cdot}81 \text{ c.c.}}$.

Therefore using formula (1), total volume = 0·81 × 10 = 8·1 c.c., where the dose factor is 1 to 10.

Using formula (2), the amount of undiluted serum required

$$= \frac{0{\cdot}81 \times 9}{10} = 0{\cdot}73 \text{ c.c.}$$

[1] The method is described in *Amer. J. Hygiene*, **18**, No. 2, Sept. 1933.

Hence for a bacterial mass of 0·9 c.c., it is necessary to add 0·73 c.c. undiluted serum and then saline to a final volume of 8·1 c.c., i.e. 6·47 c.c. saline.

After mixing thoroughly, the bacteria and diluted serum were placed in the incubator at 37° C. for 4 hours with occasional stirring and then in the refrigerator overnight. The bacteria were removed by centrifuging and the supernatant serum, already diluted 1 : 10, was used for agglutination tests with final serum dilutions of 1 : 25 to 1 : 2000 as in Dreyer's procedure.

Types of co-agglutination

From my personal records, co-agglutination has been observed in active enteric infections in persons who were without history of past enterica infection or anti-typhoid (T.A.B.) inoculation. It appears to be of two types: (*a*) where the phenomenon is present in the first sample of serum examined from the patient, and (*b*) where the phenomenon develops during the course of the disease.

(*a*) *Where the phenomenon is present in the first serum sample tested*

Example i. Co-agglutination occurring between the "O" agglutinins of *B. typhosus* and *B. paratyphosus* B, with co-existing "H" agglutinins for *B. typhosus* (in a case of typhoid fever) or for *B. paratyphosus* B (in a case of paratyphoid B fever).

This is by far the commonest example met with, and the case of Mrs A.D., aged 42 years, may be cited as an illustration (Table I). This patient became ill on 7 October 1934 and was admitted to hospital on 14 October, without a diagnosis being made. The original source of the infection could not be traced, but it is clear that the mother contracted the disease first and communicated the infection to four of her children and to a neighbouring cousin. About 2 weeks after the mother took sick, four other members of the family, whose ages ranged from 7 to 14, began to show similar symptoms and were admitted to hospital on 28 October. Blood samples were taken for the first time on 29 October when a suspicion of typhoid fever arose. Positive serological results

Table I. *Showing Ty "H", Ty "O" and* Aertrycke *"O" titres in seven samples taken during the course of typhoid fever*

Serum no.	Date when blood samples taken in 1934	End-titres				Reduced titres			
		Ty "H"	Ty "O"	PB "H"	Aer. "O"	Ty "H"	Ty "O"	PB "H"	Aer. "O"
T 12	30 Oct.	1 : 2500 (2)	1 : 200 (3)	–	1 : 250 (2)	833·3	11·8	–	50
T 21	7 Nov.	1 : 2000 (2)	1 : 200 (2)	–	1 : 100 (4)	666·7	11·8	–	20
T 33	13 Nov.	1 : 2000 (3)	1 : 200 (2)	–	1 : 100 (3)	666·7	11·8	–	20
T 45	21 Nov.	1 : 1000 (2)	1 : 200 (2)	–	1 : 100 (3)	333·3	11·8	–	20
T 69	27 Nov.	1 : 2000 (2)	1 : 250 (2)	–	1 : 100 (4)	666·7	14·7	–	20
T 75	4 Dec.	1 : 1000 (2)	1 : 500 (2)	–	1 : 100 (4)	250·0	29·4	–	20
T 82	13 Dec.	1 : 500 (2)	1 : 250 (3)	–	1 : 100 (3)	125·0	20·8	–	20

(2), (3), (4), signify strength of reactions as + +, + + +, etc. – implies absence of agglutination in 1 : 25 dilution.

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on the four children led to a blood sample being taken from Mrs A.D. on 30 October. Six subsequent blood samples were submitted at approximately weekly intervals and the agglutination titres determined.

Example ii. Co-agglutination occurring between the "O" agglutinins of *B. typhosus* and *B. paratyphosus* B, "H" agglutinins for *B. typhosus* or *B. paratyphosus* B being *absent*.

This is not commonly found: only five examples have been noted during the past 4 years.

G.D., male, aged 11 years, took sick about 1 November 1934, 3 weeks after his mother (Mrs A.D. of Ex. i) and was admitted to hospital on 4 November. In all, six samples of his blood were examined at weekly intervals, but reactions were obtained only with the somatic suspensions of *B. typhosus* and *B. aertrycke*. During his stay of 7 weeks in hospital, his temperature did not rise very high: the range was from 99 to 102° F. during the month of November. The illness was not severe and the patient was discharged on 23 December. His physician regarded him clinically as a case of typhoid fever, although typical rose spots were not observed and the specific organism was not isolated from the blood or faeces. In addition to the agglutination tests recorded in Table II his serum was examined with flagellated suspensions of *B. paratyphosus* A, *B. paratyphosus* C, *B. aertrycke* and *B. enteritidis* (Gaertner) and also with *B. dysenteriae* (Sonne) with negative results.

Table II. *Showing only Ty "O" and* Aertrycke *"O" titres in six samples taken during the course of typhoid fever*

Serum no.	Date when blood samples taken in 1934	End-titres				Reduced titres			
		Ty "H"	Ty "O"	PB "H"	Aer. "O"	Ty "H"	Ty "O"	PB "H"	Aer. "O"
T 20	7 Nov.	–	1 : 200 (2)	–	1 : 200 (2)	–	11·8	–	40
T 35	13 Nov.	–	1 : 250 (3)	–	1 : 500 (2)	–	14·7	–	100
T 46	21 Nov.	–	1 : 500 (3)	–	1 : 500 (3)	–	29·4	–	100
T 70	27 Nov.	–	1 : 500 (2)	–	1 : 500 (2)	–	29·4	–	100
T 76	4 Dec.	–	1 : 250 (3)	–	1 : 250 (3)	–	14·7	–	50
T 83	13 Dec.	–	1 : 500 (2)	–	1 : 250 (2)	–	41·7	–	50

Example iii. Co-agglutination occurring between both the "H" and "O" agglutinins of *B. typhosus* and *B. paratyphosus* B. This is a rare occurrence in my experience.

Mrs G.A., aged 57 years, was admitted to hospital on 26 October 1933 in a jaundiced condition with a history of severe headaches, fever and pain in the right hypochondriac region. These symptoms had persisted for about 4 weeks prior to admission and epistaxis had occurred five times. She had suffered from an attack of jaundice 2 years before. A history of previous enteric infection or of T.A.B. inoculation could not be elicited. She was discharged on 22 November and readmitted 10 days later. Cholecystotomy was performed on 4 December, several small gall stones removed and the gall bladder drained. During the course of her illness four blood samples were tested as recorded in Table III.

B. paratyphosus B was isolated from the faeces and from the bile after operation.

Table III. *Showing co-agglutination between "H" and "O" agglutinins in a case of paratyphoid B fever*

Serum no.	Date when blood samples taken in 1933	End-titres				Reduced titres			
		Ty "H"	Ty "O"	PB "H"	Aer. "O"	Ty "H"	Ty "O"	PB "H"	Aer. "O"
N 4	10 Oct.	1: 1000 (2)	1: 250 (2)	1: 2000 (2)	1: 250 (4)	125	22·7	500	41·7
N 7	7 Nov.	1: 500 (2)	1: 100 (2)	1: 2000 (2)	1: 250 (2)	62·5	9	500	41·7
N 42	16 Nov.	1: 250 (2)	1: 50 (2)	1: 1000 (2)	1: 100 (2)	31·3	4·5	250	20
N 58	21 Nov.	1: 250 (2)	1: 50 (2)	1: 1000 (2)	1: 100 (2)	31·3	4·5	250	20

(*b*) *Where the phenomenon is absent in the first sample of serum tested but develops during the course of the disease*

Example i. Co-agglutination between the "H" agglutinins of *B. paratyphosus* B and *B. typhosus* occurring in the third sample tested.

V.C., male, aged 19 years, was admitted to hospital on 1 August 1934 with a complaint of general malaise and loss of appetite of 8 days' duration. This was followed by pain in the left lower abdomen and across the back, stiffness in the neck and chills. Rose spots were noticed on the abdomen on 31 July. *B. paratyphosus* B was isolated from the blood and faeces. The titres of four blood samples taken during the patient's illness are given in Table IV.

Table IV. *Showing development of co-agglutination between "H" agglutinins in a case of paratyphoid B fever*

Serum no.	Date when blood samples taken in 1934	End-titres				Reduced titres			
		Ty "H"	Ty "O"	PB "H"	Aer. "O"	Ty "H"	Ty "O"	PB "H"	Aer. "O"
R 85	31 July	–	–	1: 250 (3)	1: 100 (3)	–	–	50	20
R 92	2 Aug.	–	–	1: 1,000 (3)	1: 100 (4)	–	–	200	20
S 8*	13 Aug.	1: 50 (3)	–	1: 100,000 (2)	1: 100 (4)	5	–	25,000	20
S 62	28 Aug.	–	–	1: 10,000 (2)	1: 100 (4)	–	–	2,000	20

* Repeated a few days later with the same result.

Example ii. Co-agglutination between the "O" and "H" agglutinins of *B. paratyphosus* B and *B. typhosus*, occurring in the third and fourth samples tested.

T.B., male, aged 21 years, was admitted to hospital on 8 September 1934, having been ill for 3 days previously. The chief symptoms were drenching sweats, persistent headaches, abdominal cramps and occasional nausea and vomiting. During the first 2–3 weeks the patient was in a more or less semi-comatose condition. After this time the symptoms gradually diminished in severity. The titres of five blood samples taken during his illness are recorded in Table V.

Table V. *Showing development of co-agglutination between "O" and "H" agglutinins in a case of paratyphoid B fever*

Serum no.	Date when blood samples taken in 1934	End-titres				Reduced titres			
		Ty "H"	Ty "O"	PB "H"	Aer. "O"	Ty "H"	Ty "O"	PB "H"	Aer. "O"
S 67	10 Sept.	–	–	–	–	–	–	–	–
S 68	12 Sept.	–	–	1:25 (3)	1:50 (2)	–	–	5	10
S 71	14 Sept.	–	1:50 (4)	1:200 (2)	1:100 (4)	–	3	40	20
S 99	3 Oct.	1:200 (2)	1:50 (2)	1:10,000 (3)	1:250 (2)	66·7	3	2000	50
T 2	12 Oct.	1:50 (2)	<1:25	1:5,000 (2)	1:100 (4)	16·7	–	1000	20

AGGLUTININ ABSORPTION TESTS

The frequency of the occurrence of co-agglutination with the somatic suspensions Ty "O" and Aertrycke (Paratyphoid B) "O" is evident in the serological results of Tables I–III. This has been a common occurrence in the examination of many sera for agglutinins to members of the typhoid-paratyphoid group of organisms and is due, no doubt, to the presence of an antigen or group of antigens widespread in the Salmonella group to which Kauffmann has given the symbol XII. Topley & Wilson (1929) suggest that "O" suspensions containing the ϕ antigen may act in this way. In order to ascertain whether this might be a factor in the frequency of the occurrence, sera were chosen at random and subjected to absorption tests.[1] The following may serve as an example of the results.

A sample of human typhoid serum, giving end-titres Ty "H" 1 : 2500 (++), Ty "O" 1 : 500 (+++) and Aertrycke (Paratyphoid B) "O" 1 : 500 (+++), was absorbed with a dense emulsion of *B. typhosus* H_{901}; similar samples of the same serum were also absorbed with *B. typhosus* O_{901} and with *B. paratyphosus* B (OB). The results of the agglutination tests of the unabsorbed and absorbed serum are given in Table VI.

Table VI. *Showing end-titres of serum from H.D., a case of typhoid fever*

Oxford antigen	Unabsorbed	Absorbed by		
		Ty H_{901}	Ty O_{901}	OB
Ty "H"	1 : 2500	1 : 100	1 : 2500	1 : 2500
Ty "O"	1 : 500	<1 : 25	<1 : 25	1 : 500
Aer. (Paratyphoid B) "O"	1 : 500	<1 : 25	<1 : 25	<1 : 25
		(1)	(2)	(3)

Table VI shows that (1) absorption with a flagellated emulsion of *B. typhosus* (Ty H_{901}) removes the agglutinins for the flagellar and somatic antigens; (2) absorption with a non-motile emulsion of *B. typhosus* (Ty O_{901}) removes the somatic agglutinins for *B. typhosus* and *B. paratyphosus* B but leaves the flagellar agglutinins untouched; and (3) absorption with a non-motile emulsion of *B. paratyphosus* B (OB) removes only the secondary agglutinins, i.e. the

[1] The agglutinin absorption technique is given on pp. 71–2.

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specific flagellar and somatic agglutinins for *B. typhosus* remain while the group agglutinins for *B. paratyphosus* B are absorbed.

If the results of absorption with emulsions Ty O_{901} and paratyphoid B (OB) are alone considered, it is clear that the serum saturated with the homologous organism (in the non-motile phase) loses not only its "primary" but also its "secondary" agglutinins. On the other hand, when the serum is saturated with the heterologous organism, for which a co-agglutinin had developed, it loses only that co-agglutinin, leaving the "primary" agglutinin unaffected. It is concluded therefore that in this serum sample from a case of typhoid fever co-agglutinins have been developed to Ty "O".

A contrast to these results was afforded when a sample of serum from a patient, who had received intravenous injections of T.A.B. vaccine, was subjected to absorption tests using the same bacterial strains and following the same technique as outlined on pp. 71–2. The end-titres of this serum were Ty "H" 1 : 1000 (+ +), Ty "O" 1 : 250 (+ + +), Paratyphoid B "H" 1 : 1000 (+ +), Paratyphoid B "O" 1 : 500 (+ +). Table VII shows the results of the agglutination tests of the unabsorbed and absorbed serum.

Table VII. *Showing end-titres of serum from J.D., inoculated intravenously with T.A.B. vaccine*

Oxford antigen	Unabsorbed	Absorbed by			
		Ty H_{901}	Ty O_{901}	HB_2	OB
Ty "H"	1 : 1000	<1 : 25	1 : 1000	1 : 1000	1 : 1000
Ty "O"	1 : 250	<1 : 25	<1 : 25	1 : 200	1 : 200
Paratyphoid B "H"	1 : 1000	1 : 1000	1 : 1000	<1 : 25	1 : 1000
Aer. (Paratyphoid B) "O"	1 : 500	1 : 500	1 : 500	<1 : 25	<1 : 25
		(1)	(2)	(3)	(4)

From these results it is seen that (*a*) absorption with a flagellated suspension of *B. typhosus* or *B. paratyphosus* B removes its own flagellar and somatic agglutinins only (cf. (1) and (3)); (*b*) absorption with a somatic suspension of *B. typhosus* or *B. paratyphosus* B removes its own somatic agglutinins only (cf. (2) and (4)).

From the results of the absorption tests on the human typhoid serum and the human serum resulting from intravenous inoculation, we may deduce that if saturation with the homologous organism (non-motile phase) removes both the primary and secondary agglutinins, we are dealing with co-agglutinins in the serum, but if only the specific somatic agglutinins are absorbed, we are dealing with a serum in which individual specific agglutinins are developed.

It seemed evident from a comparison of the agglutinin absorption tests on a human typhoid serum showing somatic co-agglutinins to Ty "O" (Table VI, p. 75) and on a human serum obtained after intravenous inoculation of T.A.B. vaccine (Table VII) that it is possible to differentiate between true co-agglutinins and agglutinins developed in response to individual antigens. To test this hypothesis, T.B.'s serum (fourth and fifth samples, S 99 and T 2, pooled in Table V, p. 75) was absorbed with a dense emulsion of *B. paratyphosus* B (HB_2); similar samples of this serum were also absorbed with

B. paratyphosus B (OB), *B. typhosus* H_{901} and *B. typhosus* O_{901}. The results of the agglutination tests of the untreated serum, at the time of the absorption test, and of the absorbed serum are given in Table VIII.

Table VIII. *Showing end-titres of T.B.'s serum, a case of paratyphoid B fever, before and after absorption*

		Absorbed by			
Oxford antigen	Unabsorbed	Ty H_{901}	Ty O_{901}	HB_2	OB
Ty "H'	1 : 100	<1 : 25	–	<1 : 25	–
Ty "O"	1 : 50	<1 : 25	<1 : 25	<1 : 25	<1 : 25
Paratyphoid B "H"	1 : 10,000	1 : 10,000	–	1 : 1000*	–
Aer. (Paratyphoid B) "O"	1 : 100	1 : 100	1 : 100	<1 : 25	<1 : 25
		(1)	(2)	(3)	(4)

The sign – means that the agglutination test was omitted, since absorption with somatic emulsions was found to have no effect on the original "H" titre of the serum (cf. Tables VI and VII).

* Further saturation with a dense emulsion of HB_2 removed the agglutinins to *B. paratyphosus* B "H".

A consideration of the "O" titres after absorption with emulsions of HB_2 and OB shows that the serum saturated with the homologous organism (whether in the motile or non-motile phase) loses not only its primary but also its secondary somatic agglutinins (cf. (3) and (4)). On the other hand, when the serum is saturated with the heterologous organism, whether in the motile (Ty H_{901}) or non-motile phase (Ty O_{901}), it loses only the co-agglutinin which had developed and leaves the primary agglutinins unaffected (cf. (1) and (2)). Therefore in this serum, co-agglutinins to Aertrycke (Paratyphoid B) "O" have been developed.

An examination of the "H" titres after absorption with emulsions HB_2 and Ty H_{901} shows that the homologous organism (motile phase) removes both the primary and secondary flagellar agglutinins, whereas the heterologous organism (motile phase) removes its own flagellar agglutinins, leaving the primary flagellar agglutinins unaffected. This means that co-agglutinins to *B. paratyphosus* B "H" have been developed in the serum.

The case of Mrs E.J.S., aged 32 years, is cited as an example of typhoid fever occurring in a patient who gave a history of previous enteric infection 14 years before. This patient had been confined to bed at home for 3 weeks before admission to hospital on 19 December 1934. During her residence in hospital, four blood samples were examined at approximately weekly intervals and all gave reactions to "H" and "O" suspensions of *B. typhosus* and *B. paratyphosus* B. *B. typhosus* was isolated by blood culture on 27 December 1934. The patient stated that she had suffered from an attack of typhoid or paratyphoid fever in 1920 and this statement was confirmed by her physician, but unfortunately a record of the agglutination titre could not be traced.

Samples of this patient's serum were absorbed with dense emulsions of *B. typhosus* H_{901} and O_{901}, *B. paratyphosus* B (HB_2) and OB. The results of the agglutination tests of the untreated and absorbed serum are given in Table IX.

Table IX. *Showing end-titres of serum from Mrs S., a case of typhoid fever, before and after absorption*

Oxford antigen	Unabsorbed	Absorbed by: Ty H_{901}	Ty O_{901}	HB_2	OB
Ty "H"	1 : 500	<1 : 25	–	1 : 250	–
Ty "O"	1 : 100	<1 : 25	<1 : 25	1 : 100	1 : 100
Paratyphoid B "H"	1 : 100	1 : 50	–	<1 : 25	–
Aer. (Paratyphoid B) "O"	1 : 100	<1 : 25	<1 : 25	<1 : 25	<1 : 25
		(1)	(2)	(3)	(4)

As before, if we consider the "O" titres after absorption with emulsions of Ty H_{901} and Ty O_{901}, the serum when saturated with the homologous organism (whether in the motile or non-motile phase) loses both its primary and secondary agglutinins (cf. (1) and (2)), whereas saturation with the heterologous organism whether in the motile (HB_2) or non-motile (OB) phase removes only the co-agglutinin which had developed and leaves the primary agglutinin unaffected.

If we consider now the "H" titres after absorption with Ty H_{901} and HB_2, we find that the homologous organism (motile phase) removes its own flagellar agglutinins, but fails to absorb the heterologous flagellar agglutinins present in low titre (1 : 100), and similarly with the heterologous organism. We may therefore deduce that the *B. paratyphosus* B agglutinins are not true co-agglutinins. It is suggested that the patient's illness 14 years ago was probably due to paratyphoid B infection and that her blood serum contained residual "H" agglutinins.

Summary

Co-agglutination between the somatic agglutinins of *B. typhosus* and *B. paratyphosus* B occurs more frequently than between the flagellar agglutinins in the sera of typhoid and paratyphoid B fever cases. These co-agglutinins may develop early or late in the course of the disease.

It is suggested that the agglutinin absorption test is capable of distinguishing whether or not true co-agglutinins have been developed to the flagellar and somatic antigens of *B. typhosus* or *B. paratyphosus* B.

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